agreement between the chemical and biological methods, the broths used in the present work did not contain appreciable amounts of streptomycin B.

When the resin was prepared by equilibrating with phosphate buffer solution at pH 7 in place of the saturated sodium bicarbonate solution, the eluates from the streptomycin broth showed apparent recoveries of 130 to 170%. The recovery of streptomycin from the adsorption column was determined from a comparison of the potency of the original broth, determined biologically, with that derived from the maltol reaction of the eluate. Inasmuch as the ultraviolet absorption of each aliquot was measured at 322 mμ before and after heating, the high recoveries obtained indicate the presence of biologically inactive substances in the broth which hydrolyze to give an absorption at 322 mμ, and, as the results obtained with the sodium bicarbonate equilibrated resin at pH 9 showed recoveries of 100%, these substances have a lower basic strength than streptomycin A.

The assay method described, employing adsorption at pH 9 and hydrolysis with 4 N sodium hydroxide, yields results which agree with the biological assay method to within ±5%. The reproducibility of a single chemical assay is ±3%.

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LITERATURE CITED

(1) Boxer, G. E., Jelinek, V. C., and Leghorn, P. M., *J. Biol. Chem.*, **169**, 153 (1947).
(2) Eisenman, W., and Bricker, C. E., Ind. Eng. Chem., Anal. Ed., **21**, 1507 (1949).
(3) McIntire, F. C., and Schenck, J. R., *J. Am. Chem. Soc.*, **70**, 1193 (1948).
(4) Schenck, J. R., Shaw, J. L., and Hargie, M. P., Abstracts of 113th Meeting, Am. Chem. Soc., p. 8C, Chicago, 1948.
(5) Titus, E., and Fried, J., *J. Biol. Chem.*, **174**, 57 (1948).

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Identification of Coupling Components in *p*-Toluene-Azoaryl Amide Dyes of Naphthol AS Series

LOUIS KOCH AND ROBERT F. MILLIGAN
H. Kohnstamm Research Laboratories, Brooklyn, N. Y.

A simple method has been developed for the identification of aminonaphthol AS type reduction products, by hydrogenating the pigment in dioxaneacetic acid with zinc dust, isolating the desired compound, and converting it to the *N*-benzoyl derivative.

THE amides resulting from the condensation of 2-hydroxy-3-naphthoic acid with aromatic primary amines constitute a very important group of coupling components known as the Naphthol AS intermediates. When treated with a diazonium salt, they give rise to a class of dyes which finds wide application in the textile and printing ink industries.

Unknown colors falling in this category are usually analyzed by reductive fission of the azo bond, followed by isolation and identification of the resulting products. This reaction normally splits the monoazo pigment into two fractions, the original amine and the amino derivative of the aryl amide, or their respective diamines or possibly triamines, if nitrated.

Procedures for the characterization of the former compounds are fairly numerous (*2–4, 6–9*), but except for the work of Battegay, Langjahr, and Rettig (*1*) on the synthesis of 1-aminonaphthol AS from 1-nitrosonaphthol AS and from phenylazonaphthol AS, and by Koch and Milligan (*4*) on the identification of unsulfonated dyes made with Naphthol AS, the chemical journals are practically devoid of analytical data regarding the latter products.

This paper, in an effort to bridge this gap, describes the hydrogenation of these colors with zinc dust, in dioxaneacetic acid, followed by isolation of the aminoaryl amides, which are subsequently converted into their *N*-benzoyl derivatives. Confirmation of the aminonaphthol AS group of scission products will be found in another report (*5*) which is based on the work of Battegay and co-workers (*1*). By a modification of their method, the 1-nitroso compounds of the known commercial Naphthol AS intermediates were successfully synthesized, and these gave, on reduction, amines identical with those obtained in this paper.

GENERAL PROCEDURE

Preparation of Dyes. Diazotized *p*-toluidine was coupled to twelve Naphthol AS intermediates, obtained from E. I. du Pont de Nemours & Company, and the resulting pigments were dried without further purification.

Preparation of Reduction Products. A finely ground 2-gram sample of coloring matter and 10 grams of zinc dust are placed in a 250-ml. Erlenmeyer flask, and the solids are suspended in a solution of 50 ml. of dioxane plus 10 ml. of glacial acetic acid. Heat is applied, and the mixture is refluxed gently until the dye particles are decolorized. The hot reaction mixture is filtered to remove insoluble matter, which is then washed with 25 ml. of dioxane, and the filtrate is acidified with 50 ml. of hydrochloric acid to precipitate the aminonaphthol AS hydrochloride.

Maximum yield of the addition product is achieved by cooling overnight, and the solid is then collected on a Büchner funnel, washed with a small volume of dioxane, and transferred to a 500-ml. extraction funnel with approximately 50 ml. of ethyl alcohol. Buffering the suspended hydrochloride with 10 ml. of a 10% sodium acetate solution liberates the free amine, which may wholly or partially dissolve in the alcohol. The mixture is diluted with 250 ml. of ether, and any precipitate that may remain is solubilized by further buffering with 200 ml. of a 2.5% sodium acetate solution. Several water washings are applied to the ether layer, which is then dried with anhydrous sodium sulfate, filtered, and evaporated to near dryness.

Resolution of the ether residue is effected with 50 ml. of hot benzene, and incipient crystallization or cloud formation of the aminoaryl amide is attained by dilution with petroleum ether. Cooling the solvent mixture overnight precipitates the desired compound as yellow to greenish crystals, which are collected and purified by dissolving in benzene and precipitating again in the afore-mentioned manner.

Occasionally, the aminonaphthol AS product cannot be dissolved in ether. When this occurs, 100 ml. of benzene and 50 ml. of xylene are added to the ether layer, after the water wash, and

Table I. Identification of Naphthol AS Type Coupling Components[a]

	Condensation Product of 2-Hydroxy-3-naphthoic Acid with	Commercial Name	Melting Point of 1-Amino Reduction Product (Uncor.), ° C.	Nitrogen Content Calcd., %	Nitrogen Content Found, %	Melting Point of Benzoyl Derivative of Reduction Product (Uncor.), ° C.	Nitrogen Content Calcd., %	Nitrogen Content Found, %
1	Aniline	Naphthol AS	190-2[b]	...	...	268-70	7.33	7.35
2	*o*-Toluidine	Naphthol ASD	162-4	9.58	9.38	232-3	6.98	6.79
3	*m*-Xylidine	Naphthol ASMX	166-8	9.15	9.08	241-2	6.83	6.88
4	*o*-Anisidine	Naphthol ASOL	161-3	9.09	8.96	228-30	6.79	6.65
5	*p*-Anisidine	Naphthol ASRL	160-2	9.09	8.93	249-50	6.79	6.56
6	*o*-Phenetedine	Naphthol ASOP	151-3	8.70	8.44	211-13	6.57	6.72
7	1-Naphthylamine	Naphthol ASBO	201-3	8.54	8.40	258-9	6.48	6.37
8	2-Naphthylamine	Naphthol ASSW	184-6	8.54	8.51	269-70	6.48	6.44
9	*m*-Nitroaniline	Naphthol ASBS	*183-5*[c]	*14.33*	*14.24*	*310-11*[d, e]	*8.38*	*8.27*
10	*p*-Chloroaniline	Naphthol ASE	160-2[f]	8.96	9.00	279-81	8.52[g]	8.55[g]
11	5-Chloro-*o*-toluidine	Naphthol ASTR	182-4	8.58	8.51	263-4	8.25[g]	8.28[g]
12	5-Chloro-2,4-dimethoxy aniline	Naphthol ASITR	215-17	7.52	7.36	245-6	5.88	5.84

[a] Mixed melting points with known products will differentiate between compounds such as 1-aminonaphthol ASD and 1-aminonaphthol ASOL.

[b] M.p. literature, 188-90° C.

[c] Diamine formed by reduction of nitro group on condensing amine.

[d] Dibenzoyl derivative.

[e] Extremely insoluble in solvents such as dioxane, glacial acetic acid, and xylene. Purified by leaching with boiling acetic acid.

[f] Showed tendency to darken and eventually decompose during repeated recrystallization.

[g] Chlorine content.

the combined solvents and insoluble matter are evaporated until only xylene remains. The aminoaryl amide, which will now be in solution, is precipitated with petroleum ether as previously described.

Preparation of *N*-Benzoyl Derivatives. The aminonaphthol AS products are refluxed with 1.5 grams of benzoic anhydride in 30 to 50 ml. of xylene for 2 hours, and cooled overnight. The *N*-benzoyl derivative separating out is filtered off, washed with alcohol, and crystallized from glacial acetic acid.

All melting points were taken with a standard 360° C. thermometer immersed in a Thiele tube filled with dioctyl phthalate. Customary precautions regarding heating rate and thermometer immersion depth were carefully followed.

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LITERATURE CITED

(1) Battegay, Langjahr, and Rettig, *Chemie et Industrie*, **11**, 453 (1924).
(2) Grandmougin, *Ber.*, **39**, 2494 (1906).
(3) Knecht, *J. Soc. Dyers Colourists*, **45**, 133 (1929).
(4) Koch and Milligan, IND. ENG. CHEM., ANAL. ED., **19**, 312 (1947).
(5) Koch and Milligan, *J. Am. Chem. Soc.*, in press.
(6) Koch, Milligan, and Zuckerman, IND. ENG. CHEM., ANAL. ED., **16**, 755 (1944).
(7) Milligan, Koch, and Zuckerman, *Ibid.*, **17**, 569 (1945).
(8) Whitmore and Revukas, *J. Am. Chem. Soc.*, **69**, 1500 (1937).
(9) Witt, *Ber.*, **21**, 3468 (1888).

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Photoelectric Determination of Atmospheric Sulfur Dioxide

Employing Dilute Starch-Iodine Solutions

MORRIS KATZ, *Defence Research Chemical Laboratories, Ottawa, Canada*

THE early methods for determining the concentration of sulfur dioxide in air were more suitable for flue gas analysis than for the low concentrations ordinarily found in urban and smelter areas.

The first method adapted to the rapid determination of small amounts of sulfur dioxide in air was that used by Marston and Wells in the investigation carried out by the Selby Smelter Commission (*5*). Measured volumes of air were drawn into a partly evacuated 24-liter aspirator bottle. A definite amount of starch solution containing sufficient iodine to bring the reagent to a desired blue color was added, and the aspirator bottle was shaken vigorously to bring the air sample in contact with the starch-iodine solution. The color of the partly bleached iodine solution was then restored by the addition of standard iodine reagent so as to match that of a blank similarly treated. Reasonably close checks were obtained against known mixtures of sulfur dioxide in air in the range of 0.80 to 3.00 p.p.m. With lower or higher concentrations the errors were as high as 20 to 25%. This method, with certain modifications, was used by Monnett (*10*) in his survey of atmospheric conditions at Salt Lake City, in the investigation by the Swain Commission (*12*), and later by McKay and Ackermann (*8*).

The preparation of stable iodine solutions is important in methods of this type, because a rapid stream of pure air passed through an aqueous iodine solution will soon weaken the strength of the solution. If, on the other hand, the vapor pressure of the iodine in solution is repressed by the addition of potassium iodide and starch, solutions may be made that are remarkably stable even after prolonged aspiration with air. Zepf and Vetter (*19*) investigated the stability of very dilute iodine solutions and showed that a certain minimum quantity of both starch and potassium iodide was necessary to eliminate loss from a 0.0001 *N* iodine solution.

A distinct advance over the method of Marston and Wells was made by Thomas (*13-17*), who developed not only a portable apparatus for the rapid measurement of small concentrations of sulfur dioxide in air but also a continuous automatic method which has become widely used in recent years. Thomas and Cross (*15*) investigated the stability and efficiency of iodine solutions for absorbing sulfur dioxide. When 28 liters of pure air were aspirated through 100 ml. of 0.0011 *N* iodine solution, containing 2 grams of potassium iodide and 1 gram of starch per liter, the loss of iodine was about 4%. Only about half of this material was caught in the second absorber. A 0.0006 *N* solution was changed only about 1% by this treatment and more dilute solutions were very stable. A 0.000075 *N* solution did not change when 400 liters of air were drawn through it.

The iodine method advocated by Thomas involved the use of an absorbing solution of 0.0005 *N* iodine, containing 1 gram of